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Short communication

Co-infection of chickens with *Eimeria praecox* and *Eimeria maxima* does not prevent development of immunity to *Eimeria maxima*

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ABSTRACT

Previous studies revealed an ameliorating effect of *Eimeria praecox* on concurrent *E. maxima* infection, such that weight gain, feed conversion ratio, and intestinal lesions were nearly identical to uninfected or *E. praecox*-infected controls. The purpose of the present study was to determine if protective immunity against *E. maxima* challenge infection developed in chickens infected with both *E. praecox* and *E. maxima*. Day-old chickens were infected with 10^3 *E. praecox*, 10^3 *E. maxima*, or a mixture of 10^3 *E. praecox* and 10^3 *E. maxima* oocysts. Chickens were then challenged at 4 weeks of age with 5×10^4 *E. praecox* or 5×10^3 *E. maxima* oocysts and clinical signs of coccidiosis were assessed 7 days post-challenge. Relative to non-challenged controls, naïve chickens or chickens immunized with *E. praecox* displayed a 32–34% weight gain depression after challenge with 5×10^3 *E. maxima* oocysts. In contrast, chickens immunized with either *E. maxima* oocysts alone or a combination of *E. praecox* and *E. maxima* oocysts displayed complete protection against lower weight gain associated with *E. maxima* challenge. Also, protection against decreased feed conversion ratio and intestinal lesions was observed in single *E. maxima*- or dual *E. maxima* + *E. praecox*-immunized chickens. These findings indicate that co-infection of chickens with *E. maxima* and *E. praecox* does not prevent development of immunity against *E. maxima* or *E. praecox* challenge.

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1. Introduction

One consistent finding in studies of avian coccidiosis is the species-specific immunity that develops after a primary infection with *Eimeria* oocysts (Allen and Fetterer, 2002; Shirley et al., 2007). The absence of appreciable cross-immunity between species is the basis for incorporating different *Eimeria* species in live oocyst vaccines. In fact, most live oocyst vaccines for use in broilers are composed of only three *Eimeria* species, namely *E. acervulina*, *E. maxima*, and *E. tenella*, because these are the predominant and/or the most pathogenic species in poultry operations. Recent studies by our group and others have found that, irrespective of whether drugs or vaccines are used to control coccidiosis, *E. praecox* is present on a high percentage of commercial poultry farms (Jenkins et al., 2006; Morris et al., 2007; Haug et al., 2008). In a recent study, *E. praecox* was shown to reduce the clinical effects of *E. maxima* infection when chickens were infected at the same time with both species (Jenkins et al., 2008). This work suggests that co-infection of chickens with two *Eimeria* species that infect similar regions of the gut, one species non-pathogenic (*E. praecox*), the other species pathogenic (*E. maxima*), elicits non-specific immunity against both *E. praecox* and *E. maxima*, thereby reducing clinical signs of coccidiosis. The purpose of the present study was to determine if *E. praecox*, in addition to its ameliorating effect on *E. maxima* infection, inhibits the development of immunity to *E. maxima*.

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2. Materials and methods

2.1. Parasites

Eimeria maxima (strain Arkansas 1) and *E. praecox* (strain North Carolina 2) were derived from field samples, single oocyst-isolated, and propagated every 2–3 months in susceptible chickens using standard procedures (Ryley et al., 1976). The purity of both strains was confirmed by species-specific polymerase chain reaction (PCR) based on ITS1 rDNA sequence (Jenkins et al., 2006).

2.2. Cross-immunity studies

The effect of *E. praecox* on development of immunity to *E. maxima* infection was carried out by infecting susceptible 1-day-old male Sexsal chickens (3 groups/treatment, 5 chickens/group, 15 chickens total/treatment) with either 10^3 *E. praecox* (Ep) oocysts, or 10^3 *E. maxima* (Emax) oocysts, or a mixture of *E. praecox* and *E. maxima* (Ep + Emax) oocysts at the above dose levels. At 28 days, chickens were challenged with either 5×10^4 *E. praecox* oocysts or 5×10^3 *E. maxima* oocysts. The timing of primary and challenge infections was based on current strategies to vaccinate chickens against coccidiosis using live oocyst vaccines and to allow sufficient time for immunity to develop (Allen et al., 2005; Chapman et al., 2005; Chapman and Rayavarapu, 2007). Controls included non-immunized and non-infected chickens (NINC), and non-immunized chickens that were challenged with either 5×10^4 *E. praecox* oocysts or 5×10^3 *E. maxima* oocysts (NIC). On day 7 post-challenge, all chickens were killed by cervical dislocation and necropsied for determining upper-middle intestinal lesion scores using standard procedures (Johnson and Reid, 1970). Body weights were obtained for all individual chickens to allow calculation of weight gain during the experimental period. Feed conversion ratio was calculated for each replicate of treatments by dividing total feed consumed by total weight gain during the experimental period. The entire study was repeated twice for a total of three studies.

2.3. Statistical analysis

Body weight gain, intestinal lesion scores, and feed conversion ratios were compared between treatment groups using Duncan's Multi-Range Comparison Test (SAS Institute, Inc., Cary, NC). Results were expressed as mean ± S.E.M. of three independent trials. Significant differences between groups were noted if $P \leq 0.05$.

3. Results

3.1. Effect of E. praecox infection on immunity to E. maxima

3.1.1. Weight gain depression

Control non-immunized groups (NIC) that were challenged with *E. maxima* oocysts displayed a significant reduction ($P < 0.05$) in average weight gain (Fig. 1A). Average weight gain was reduced by an equal amount ($P < 0.05$) in chickens immunized with *E. praecox* (Ep) alone and challenged with *E. maxima* (Fig. 1A). In contrast, complete protection against reduced weight gain was observed in chickens immunized with either *E. maxima* (Emax) alone or a mixture of *E. praecox* and *E. maxima* (Ep + Emax) showing no significant difference from non-challenged controls (NINC, $P > 0.05$, Fig. 1A).

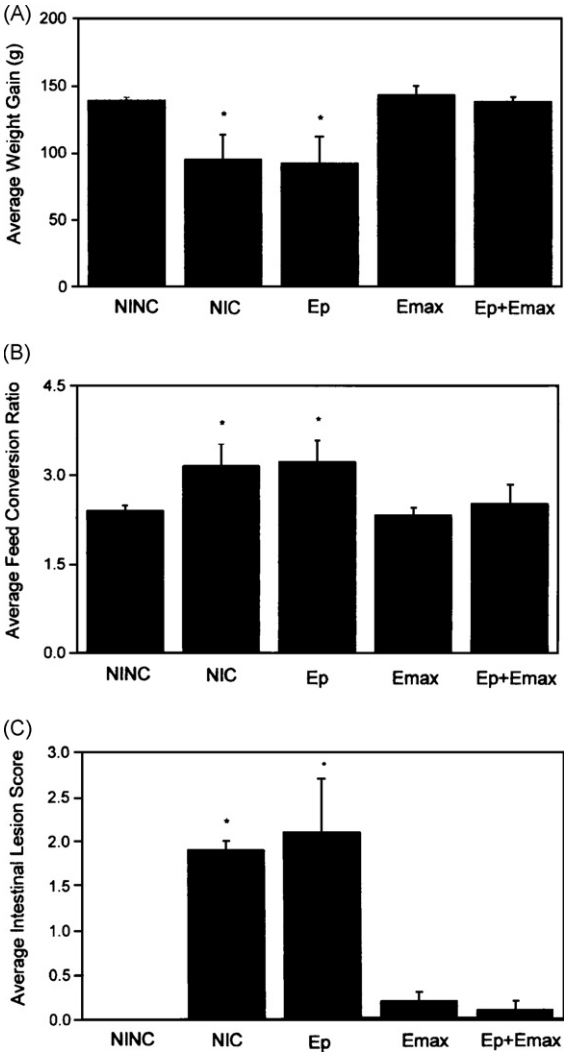

Fig. 1. Average weight gain (A), feed conversion ratio (B), and intestinal lesion scores in middle-upper intestine (C) at 7 days post-*Eimeria maxima* challenge in chickens immunized with 10^3 *Eimeria praecox* (Ep) oocysts or 10^3 *E. maxima* (Emax) oocysts or with 10^3 each *E. praecox* and *E. maxima* (Ep + Emax). NIC, non-immunized, *E. maxima*-challenged controls; NINC, non-immunized, non-challenged controls.

3.1.2. Feed conversion ratios (FCR)

The average feed conversion ratio (FCR) in non-challenged controls (NINC) was 2.40 ± .09, whereas average FCR in non-immunized groups that were challenged with *E. maxima* oocysts (NIC) was 3.16 ± 0.36 (Fig. 1B), representing a significant increase over controls ($P < 0.05$). A similar increase in average FCR was observed in chickens immunized with *E. praecox* (Ep) alone and challenged with *E. maxima* (Fig. 1B). However, average FCR in *E. maxima*-challenged

chickens that had been immunized at 1 day of age with either *E. maxima* (Emax, FCR = 2.34 ± 0.12) or with a combination of *E. praecox* and *E. maxima* (Ep + Emax, FCR = 2.53 ± 0.33) was similar to non-challenged controls (NINC, Fig. 1B).

3.1.3. Intestinal lesion scores

The average intestinal lesion score in non-immunized chickens (NIC) that were challenged at 4 weeks of age with *E. maxima* oocysts was 1.9 ± 0.1 (Fig. 1C). A similar average lesion score was observed in *E. praecox*-immunized chickens (Ep) that had been challenged with *E. maxima* oocysts (2.1 ± 0.6). However, intestinal lesions were greatly reduced ($P < 0.05$) in *E. maxima*-challenged chickens that had been immunized with either *E. maxima* (Emax, 0.2 ± 0.1) or with a combination of *E. praecox* and *E. maxima* (0.1 ± 0.1) (Ep + Emax, Fig. 1C).

3.2. Effect of E. maxima infection on immunity to E. praecox

3.2.1. Weight gain depression

Control non-immunized groups that were challenged with *E. praecox* oocysts displayed lower, yet insignificant ($P > 0.05$), average weight gain compared to non-challenged controls (NINC, Fig. 2A). Weight gain was also reduced in chickens immunized with *E. maxima* alone and challenged with *E. praecox* (Emax, Fig. 2A). Average weight gain in *E. praecox*-challenged chickens that had been immunized by oral inoculation with *E. praecox* (Ep) alone or with a combination of *E. praecox* and *E. maxima* (Ep + Emax) was greater than non-immunized, *E. praecox*-challenged controls (Fig. 2A).

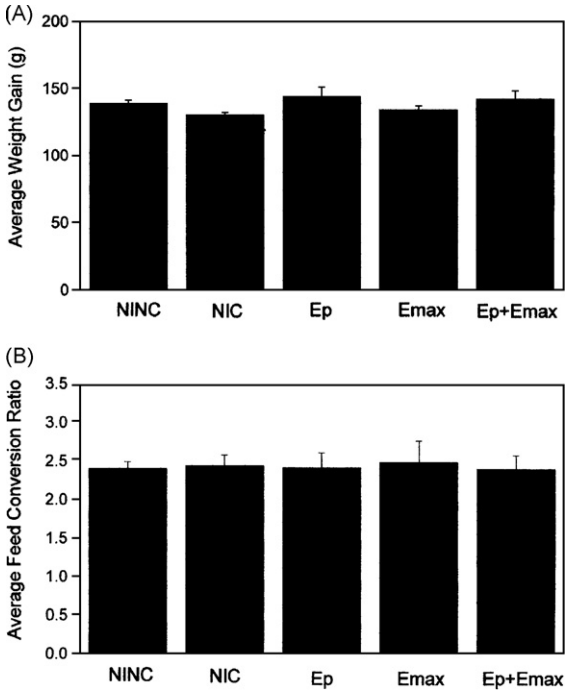

Fig. 2. Average weight gain (A) and feed conversion ratio (B) at 7 days post-*Eimeria praecox* challenge in chickens immunized with 10^3 *Eimeria praecox* (Ep) oocysts or 10^3 *E. maxima* (Emax) oocysts or with 10^3 each *E. praecox* and *E. maxima* (Ep + Emax). NIC, non-immunized, *E. praecox*-challenged controls; NINC, non-immunized, non-challenged controls.

3.2.2. Feed conversion ratios (FCR) and intestinal lesions

No significant differences were observed in FCR between non-immunized *E. praecox*-challenged (NIC) and non-challenged control groups (NINC, $P > 0.05$, Fig. 2B) reflecting the low pathogenicity of *E. praecox*. Also, regardless of prior exposure to *E. praecox* and/or *E. maxima*, no significant increase in average FCR was observed in any group ($P > 0.05$). Similarly, negligible lesions were observed in *E. praecox*-infected groups, regardless of whether chickens were not immunized or were immunized with *E. praecox* and/or *E. maxima* (data not shown).

4. Discussion

The present study demonstrated that immunity to *E. praecox* or *E. maxima* challenge develops in chickens that were infected at 1 day of age with both *Eimeria* species. Although *E. praecox* can ameliorate clinical signs associated with *E. maxima* (Jenkins et al., 2008), it does not appear to interfere with development of immunity to *E. maxima*. While *E. praecox* appears to be less pathogenic than other *Eimeria* species, it can affect weight gain at high challenge doses, and immunity to *E. praecox* readily develops in chickens (Gore and Long, 1982). This study provides evidence that acquired resistance to *E. praecox* challenge is similar to immunity against *E. maxima*. That is, co-infection of chickens at 1 day of age with *E. maxima* and *E. praecox* does not prevent development of immunity to *E. praecox*.

The practical implications of this study and previous work showing an ameliorating effect of *E. praecox* on clinical signs associated with *E. maxima* are several-fold. One is that including *E. praecox* in a live oocyst vaccine may not only induce immunity to *E. praecox*, but also lessen the negative effects caused by vaccine strains of *E. maxima* (Williams, 1998). Second, the high prevalence of *E. praecox* in the field (Jenkins et al., 2006; Morris et al., 2007; Haug et al., 2008) may reflect persistent drug-resistance in this species, thus alternative control measures, such as vaccination, may be warranted. It remains unknown what impact *E. praecox* has on broiler productivity relative to other more pathogenic *Eimeria* species. A number of authors have shown that at high oocyst doses *E. praecox* has significant impact on weight gain and feed conversion efficiency (Long, 1968; Gore and Long, 1982; Jorgensen et al., 1997; Williams, 1998). Whether chickens are exposed to these levels of *E. praecox* in the field is unknown.

These findings may also provide some insight on the nature of immunity to *E. maxima*, and possibly other *Eimeria* as well, and indicate potential ways to reduce clinical effects of coccidiosis during a primary infection. Our research findings suggest that inducing a local non-specific immune response in a region of the chicken intestine invaded by a particular *Eimeria* species may reduce the clinical signs of coccidiosis, but still allow for limited replication of the parasite, and development of protective immunity to challenge infection.

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